High Production of Hyaluronic acid by *Streptococcus zooepidemicus* in Cheese Whey

Isabel R. Amado[1], José A. Vázquez[2], Lorenzo Pastrana[1] and José A. Teixeira[3]

[1]Departamento de Química Analítica y Alimentaria, Universidad de Vigo, Ourense (Spain)
[2]Grupo de Reciclado y Valorización de Materiales Residuales, IIM-CSIC, Vigo (Spain)
[3]Institute for Biotechnology and Bioengineering, CEB (Universidade do Minho), Braga (Portugal)

Background

Hyaluronic acid (HA) is a high molecular mass biopolymer composed of dymeric units of N-acetyl glycosamine and glucuronic acid (Fig. 1). It is present in animal tissues (cartilage, umbilical cord, synovial fluid, vitreous humour) and in bacterial cell walls [1].

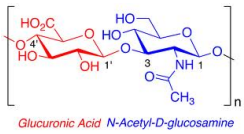

Figure 1. Hyaluronic acid

HA is a high value product with an increasing level of demand in the clinical and pharmaceutical sectors due to its high MW and interesting viscoelastic and rheological properties [2]. Interest in the production of HA by fermentation has focused scientific research in the last years. However, the formulation of cost-effective culture media must be addressed for maintaining the commercial competitiveness of this bioproduction.

Whey is the main by-product of the cheese manufacturing industry (85–95% of the milk volume), consisting on the watery portion that is formed during the coagulation of milk casein [3] (Fig. 2). Currently this wastewater represents an environmental problem, but due to its high nutritional content is being considered as a resource for different biotechnological applications.

Figure 2. Cheese producing industry

In this study two culture media containing hydrolyzed (HCW) or unhydrolyzed cheese whey (CW) were formulated and assessed as a protein source for the microbial production of HA using *Streptococcus zooepidemicus*.

Culture media preparation

CW was hydrolyzed with Alcalase 2.4 L (Novozyme Nordisk, Bagsvaerd, Denmark) at 45°C under orbital agitation (100 rpm) for 2 h. The enzyme/substrate ratio was 9.6 U/kg of soluble protein in the cheese whey.

After pre-treating CW and HCW (Fig. 3), culture media were prepared by adding glucose and salts at the same level as the complex medium (CM), being the final composition shown in Table 1.

Table 1. Culture media composition in g/L.

	CW	HCW	CM
Glucose	50.0	50.0	50.0
Yeast extract	5.0	5.0	5.0
Tryptone	–	–	15.0
KH_2PO_4	2.0	2.0	2.0
$MgSO_4.7H_2O$	0.5	0.5	0.5
$(NH_4)_2SO_4$	0.5	0.5	0.5

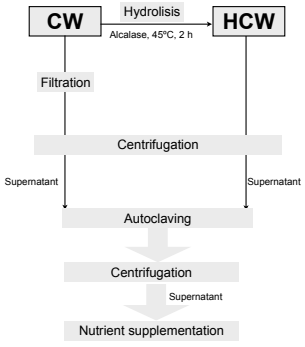

Figure 3. Scheme illustrating culture media preparation

Culture conditions

A 2-vessel glass 5 l-bioreactor (Biostat B, Braun Sartorius) with a working volume of 4.5 L was utilised for HA production. The culture was carried out at 37°C, 500 rpm of agitation, 1 vvm of aeration and controlled pH (6.7).

Sampling and analytical methods

Samples were taken from the bioreactor and incubated with a 10% of 5% (w/v) SDS for 10 min [4]. Then the biomass was removed by centrifugation at 15000 ×g for 15 min and the optical density (OD) measured at 700 nm. The content of reducing sugars, glucose, LA, proteins and HA was determined in the supernatant.

Results

According to our results, the highest biomass and hyaluronic acid productions were achieved in CW compared to HCW and CM (Figure 4). In CW medium, best-fit values from data modelling showed maximal concentrations of 4.77±0.26, 4.06±0.15 and 51.5±4.9 g/L for biomass, HA and lactic acid (LA), respectively (Table 2). Besides, the use of CW as a protein source reduced the lag phase of biomass and lactic acid productions ($p<0.05$) compared to the control.

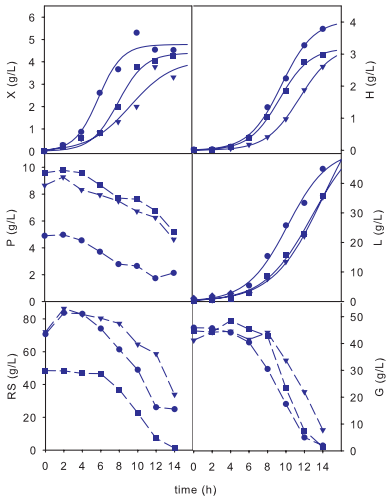

Figure 4. Batch cultures of *Streptococcus zooepidemicus* in hydrolyzed (HCW; ▼), unhydrolyzed cheese whey (CW; ●) media and complex medium (CM; ■). Continuous lines are the fittings of the experimental results (points) according to equations reported in [4]. X: biomass; H: hyaluronic acid; P: protein; L: lactic acid; RS: reducing sugars and G: glucose.

Although the lag phse of HA production using CW was similar to that of CM, the rate of biomass, HA and LA were higher compared to the commercial culture medium (Table 2).

Table 2. Estimations of biomass, hyaluronic and lactic acid productions in batch cultures of *Streptococcus zooepidemicus* in hydrolyzed (HCW), unhydrolyzed cheese whey (CW) media and complex medium (CM).

	CW	HCW	CM
Biomass (X)			
K (g/L)	4.77 ± 0.66	4.10 ± 2.27	4.39 ± 0.58
ν_X (g/L.h)	0.93 ± 0.55	0.45 ± 0.25	0.75 ± 0.28
λ_X (h)	3.31 ± 1.67	4.85 ± 1.01	5.10 ± 1.15
Hyaluronic acid (H)			
H (g/L)	4.06 ± 0.38	3.27 ± 0.20	3.19 ± 0.25
ν_H (g/L.h)	0.58 ± 0.08	0.45 ± 0.02	0.48 ± 0.07
λ_H (h)	5.99 ± 0.50	7.87 ± 0.15	6.00 ± 0.47
Lactic acid (L)			
H (g/L)	51.5 ± 12.7	71.88 ± 35.1	58.20 ± 36.3
ν_H (g/L.h)	5.85 ± 1.14	6.57 ± 1.97	5.59 ± 1.70
λ_H (h)	5.79 ± 0.90	8.65 ± 1.48	7.66 ± 1.68

Conclusions

CW culture broth provides high HA yields and is a cost-effective alternative for the commercial production of HA by *Streptococcus zooepidemicus*. Besides, this approach contributes to the valorisation and depuration of cheese whey which is currently an environmental problem.

Future work

CW culture broth should be optimised by reducing nutrient supplementation in order to reduce even more HA producing costs. Also other cultivation strategies should be addressed towards an increased HA production using CW.

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